X-PEEM studies of protein interactions with patterned polymer surfaces

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INTRODUCTION

Synthetic biomaterials are widely used in medical applications. However, in many cases current materials are not optimum since they do not interact with cells through specific proteins or peptides; rather the interaction with the body is mediated through passive adsorption of a disorganized protein monolayer. It has been hypothesized that mis-recognition of this disorganized adsorbed protein layer by surrounding cells leads to the classic foreign body reaction and device encapsulation [1]. Next generation biomaterials, so-called **"engineered biomaterials,"** are being designed in which the surface contains specific bio-recognition moieties which control the biological response of the host. We are exploring the use of X-ray photoelectron emission microscopy (X-PEEM) [2] to monitor methods for preparing patterned functionalized biomaterial surfaces, and to investigate the specificity of the interaction of model surfaces with key proteins. So far this project has focused mainly on validating the methodology and providing feedback for pattern preparation, although some studies of the interaction of proteins with patterned surfaces have been carried out. It is a complement to a STXM / X-PEEM project whose goal is to image proteins on polyurethanes used for blood contact medical applications [3].

RESULTS AND DISCUSSION

Biological signaling agents can be deposited on a surface with spatial precision and fidelity through microcontact printing (μCP) [4] which uses an elastomeric template to transfer protein molecules to a surface of interest. It is a versatile and simple means of preparing surface protein patterns. μCP is combined with thiol-Au self assembly chemistry either prior to or following μCP to form or fill in patterns on surfaces. To test the reliability of the surface patterning method, we use highly specific bio-recognition pairs, such as the biotinylated ferritin - streptavidin couple, to probe the quality of the patterned surface. Such structures are then investigated with elemental (Fe 2p) and molecular (C 1s and N 1s) speciation using NEXAFS microscopy recorded with the BL 7.3.1 X-PEEM.

Here we describe one example of this work. A gold coated native oxide silicon wafer was stamped with polyethylene glycol (PEG) in nominal 2 μm x 2 μm lanes. This sample was then backfilled over 2-days with 1,1,2,2-perfluorodecanethiol (FC). It was dried and then exposed to streptavidin (SA) overnight. Excess SA was rinsed off with deionized water (without the sample breaking the solution-air interface until heavily diluted) and then blown dry. The X-PEEM measurements were made within 2 days of the preparation.

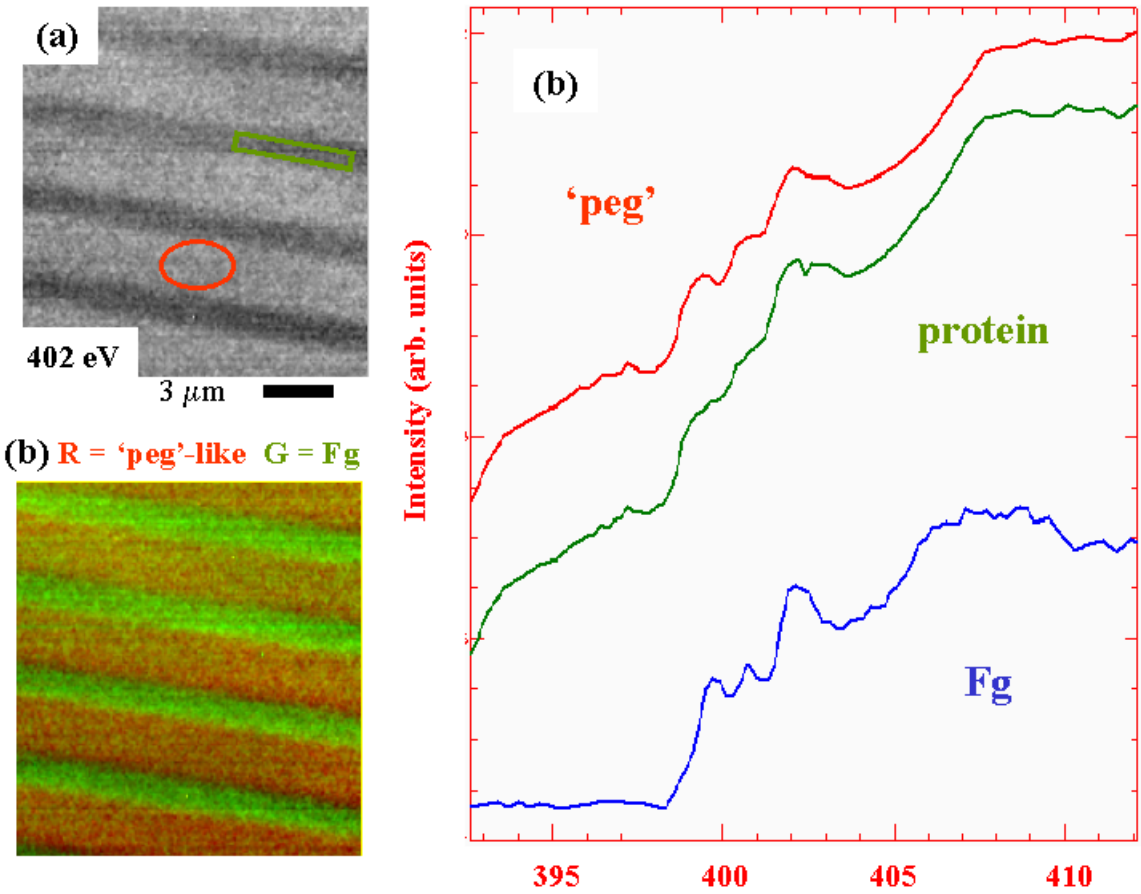

Fig. 1 (a) X-PEEM image at 402 eV. (b) N 1s NEXAFS spectra extracted from the image sequence at the regions indicated by colored lines, compared to the background subtracted N 1s spectrum of fibrinogen. (c) color coded composite image (red = 'peg'-like; green = 'Fg'-like), derived from SVD analysis of N 1s image sequence.

Fig. 1 plots the image recorded at 402 eV (peak of amide signal), the N 1s spectra extracted from the narrow and wide striped regions, and a color coded composite of the protein and polymer maps derived by a pixel-by-pixel linear regression [5]. The spectral models used were the known spectrum of protein, and a computed (structureless) curve for the PEG/FC polymers, neither of which contain nitrogen. The color coded map suggests there is greater amounts of protein in the narrow lanes. However both the narrow and wide lanes exhibit the N 1s NEXAFS spectrum of a protein, and really differ mainly in the S:B ratio. It would appear that streptavidin is adsorbed over the whole surface with perhaps some excess in the narrower lanes, which are likely thinner than the wide lane, less proud of the surface, and thus give less intense signal due to PEEM topographic contrast. The absence of strong selectivity in the protein adsorption may be explained by examining the F 1s X-PEEM results for the surface prior to streptavidin exposure (**Fig. 2**). This shows that the backfill reaction of the PEG-stamped surface has resulted in coverage of the whole surface with fluorocarbon, not just the PEG-free regions, as had been expected. This example indicates that NEXAFS microscopy recorded with X-PEEM on thin, protein coated polymer layers are sensitive to details of protein adsorption on patterned organic surfaces. The X-PEEM results are being combined with XPS, AFM, functionalized probe SPM [6] and non-spatially resolved NEXAFS to provide analytical support for a systematic program of biomaterials optimization.

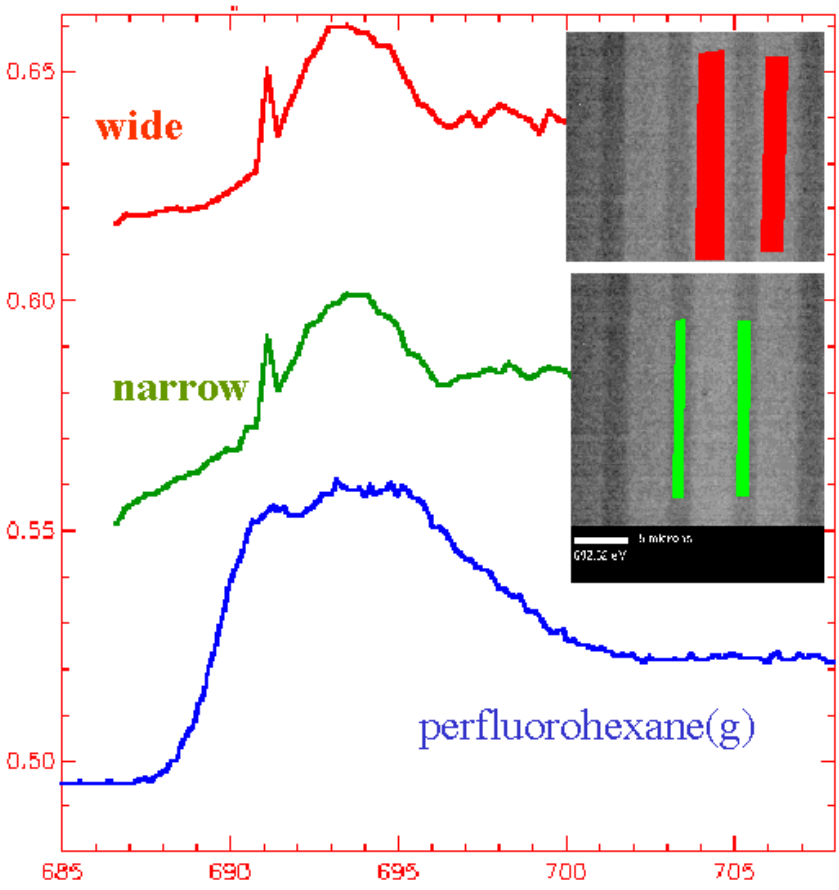

Fig. 2 F 1s NEXAFS spectra extracted from the indicated color-coded regions of an X-PEEM image sequence recorded from a PEG-FC patterned Au surface. The similarity of F 1s signal in the narrow and wide regions indicates the chemical reaction used to attempt to prepare alternate PEG and FC stripes likely resulted in both the PEG stripes and the unexposed Au being coated with fluorocarbon.

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